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Aflatoxin and fumonisin contamination of corn (maize, *Zea mays*) hybrids in Arkansas

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Abstract

A severe infestation by aflatoxin-producing fungi diminished food quality of southern United States corn (maize) in 1998. Corn hybrids (65) naturally infected with *Fusarium* spp. and *Aspergillus* spp. were evaluated from 1998 to 2001 for resistance to mycotoxin contamination. Kernel corn samples were assayed at harvest for aflatoxins and fumonisins. In 1998, samples from all hybrids exceeded 20 ppb aflatoxin (mean levels: 21–699 ppb) and 2 ppm fumonisins (mean levels: 23–79 ppm), the maximum levels permitted by United States Food and Drug Administration guidelines. Samples from hybrids planted in the same and other locations in Arkansas in 1999 and 2001 were shown by similar methods to contain aflatoxin levels ranging from not detected to 255.3 ppb and fumonisin levels from 0.3 to 83.6 ppm. The fumonisin levels in 2001 were very high in all hybrids, ranging from 8 to 83.6 ppm while aflatoxin levels were low ranging from <5 in most hybrids to 131 ppb. The presence of aflatoxin B_1 and B_2 in samples was confirmed by thin layer chromatography and liquid chromatography/mass spectrometry and fumonisins B_1, B_2, B_3, B_4 and C_4 by liquid chromatography/mass spectrometry. During the period studied, a positive correlation was observed between aflatoxin and fumonisin levels, indicating that natural infection with *Fusarium* spp. did not appear to protect against aflatoxin production.

Keywords: Aflatoxin; Fumonisin; Corn hybrids; Maize; Mycotoxins; Heat stress; Drought; Insect damage; Ear rots; *Aspergillus*; *Fusarium*

1. Introduction

Corn (*Zea mays* L.) is a major crop in the southern United States, where it plays an important role in the economy in rotation with cotton or soybeans, and in animal feed, alcohol fermentation and direct human consumption. However, corn kernels are subject to infection by a variety of toxigenic fungi (Francis and Burgess, 1975; Marasas et al., 1981; Zummo and Scott, 1992; Widstrom, 1996; Abdullah et al., 1998; Marin et al., 1998a; Cardwell et al., 2000), most commonly *Aspergillus flavus*, *Fusarium verticillioides* (syn. *F. moniliforme*) and *F. proliferatum* (Marasas et al., 1979; Gonzalez et al., 1995; Chulze et al., 1996; Picco et al., 1999; Cardwell et al., 2000). The aflatoxins and fumonisins produced by these fungi have an important economic impact on the grain industry. The risks of fumonisins to human and animal health (Marasas et al., 1988; Gelderblom et al., 1988, 1996, 2002; Harrison et al., 1990; Tolleson et al., 1996; National Toxicology Program, 1999) prompted the United States Food and Drug Administration to propose a guideline to allow a maximum content of 2 ppm in corn and corn products for human consumption (United States Food and Drug Administration, 2001). Aflatoxin has long been monitored by the United States Food and Drug

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Administration, and a level of 20 ppb has been set as the limit for corn contamination with aflatoxin (United States Food and Drug Administration, 2000).

Fungal growth and toxin production in corn have been found to depend on several interacting factors that stress corn plants (Payne, 1992). Stress factors include low moisture content of the soil, high daytime maximum temperatures, high nighttime minimum temperatures, and nutrient-deficient soils (Lillehoj et al., 1980; Miller et al., 1983; Widstrom et al., 1990; Abramson, 1998; Abbas et al., 2002). The present study is part of a systematic effort by these laboratories to understand the environmental, cultural, genetic and husbandry-related factors that result in economically important contamination of crops by the mycotoxins aflatoxin and fumonisin. At present, economically disastrous events like the widespread aflatoxin contamination in the midwestern and southern United States 1998 (Windham and Williams, 1999; Windham et al., 1999) are unpredictable even in the short term, because the factors that cause them are not fully understood. The long-range goal of this research program is to evaluate under field conditions the impact of various factors that affect aflatoxin and fumonisin contamination levels in corn. Most of these factors have been identified in laboratory- and greenhouse-based studies, so the strategy used in the research program has been to evaluate the impact of progressively more factors on aflatoxin and fumonisin levels in corn grown under field conditions in the southern United States. Each step in this process is intended to move the knowledge base further from the laboratory and closer to the reality of large-scale commercial production. In the present study, the effects of heat stress on aflatoxin and fumonisin contamination at natural infection levels of *A. flavus* were investigated by comparing mycotoxin contamination in conventional and Bt hybrid corn lines in a high heat stress growing season (1998) with contamination in subsequent moderate stress growing seasons (1999 and 2001).

2. Materials and methods

2.1. Corn growing conditions

In 1998, 21 corn hybrids, including four Bt-corn hybrids, were planted on May 14 and harvested on September 25 at the Cotton Branch Experimental Station, Marianna, Arkansas, USA. In 1999, 29 corn hybrids, including 12 Bt hybrids, were planted on May 14 and harvested on September 10 at the same location. In 2001, nine corn hybrids were planted on May 8 and harvested on October 8 at the same location. The same nine corn hybrids were also planted at the Pine Tree Experiment Station, Colt, Arkansas, USA, on May 8 and harvested on October 8. In all years fertilization, weed control and irrigation were used to minimize contributions from nutrient, population and drought stresses. Furrow irrigation of plots was done with 11 irrigations in 1998, eight in 1999, and six in 2001. Hybrids were grown in a randomized complete block design with four replications. Plots were managed using current Cooperative Extension Service guidelines in Arkansas with respect to weed control and fertilization. In these studies, it has been assumed that differences in toxin content of kernels at harvest among various corn hybrids grown in the same area under the same conditions reflect differences in resistance to the toxin-producing disease that is either inherent, resulting from selective breeding or acquired with a recombinant gene added to control a vector of the disease. Previous studies (Abbas et al., 2002) have compared mycotoxin production by corn hybrids from a variety of sources using artificial inoculation of the ears with cultured fungus, whereas the present study examines corn hybrids naturally infected in the field with *A. flavus* and *Fusarium* species. In 1998 levels of insect damage, Aspergillus ear rot, and Fusarium ear rot were determined in samples, as well as mycotoxin contamination. In 1999 and 2001, only mycotoxin contamination was determined.

2.2. Collection of corn samples

Random ears (10–20) were hand-harvested from each plot at approximate grain maturity for mycotoxin analysis. Husks were removed immediately and ears stored dry in paper bags. For the crop of 1998, a representative sample of ears from each hybrid was measured, and visually assessed for the percent of kernels with (i) obvious insect damage, (ii) Aspergillus rot, and (iii) other rots, primarily due to *Fusarium* sp. Ear assessment was made as soon as possible after harvest. Aspergillus rot and Fusarium rot were confirmed microscopically or by culture of the respective pathogens according to the methods of Abbas et al. (1988, 1992, 2002, 2004). Ears were machine-shelled, and grain samples of at least 1 kg from each row were mixed twice in a sample splitter. Samples (1 kg) were ground using a Romer mill (Union, MO, USA) and one-third selected for extraction as described below.

2.3. Extraction and clean-up of mycotoxins from corn samples

Extraction and clean-up of mycotoxins from corn samples was carried out as described by Abbas et al. (2002). Briefly, sub-samples of ground field corn (20 g) were extracted with 100 mL of methanol/water, 70/30, filtered, evaporated to dryness under a stream of nitrogen, and stored at 5 °C until subjected to clean-up and high-performance liquid chromatography (HPLC)

analysis. Clean-up of aflatoxin samples was accomplished after reconstitution in 10 mL of 70% methanol by absorption on conditioned aflatoxin affinity columns (Neogen Corp., Lansing, MI, USA), which were washed with two 10 mL volumes of 25% methanol in water, eluted with 2 mL of 100% methanol, and evaporated to dryness. Clean-up of fumonisin samples reconstituted in 10 mL of 70% methanol utilized adsorption on conditioned Bond-Elute SAX single-use columns (Chrom-Tech, Inc., Apple Valley, MN, USA). The columns were washed with 8 mL of 75% methanol and 3 mL of 100% methanol, then eluted with 14 mL of 0.5% acetic acid in methanol and evaporated to dryness.

2.4. Enzyme-linked immunosorbant assay (ELISA) analysis of aflatoxins and fumonisins

Commercially available quantitative ELISA assay kits (Neogen Corp., Lansing, MI, USA) were used for measuring the presence of total aflatoxins and total fumonisins according to the manufacturer's instructions.

2.5. Liquid chromatography/mass spectrometric analysis of mycotoxins

All solvents were HPLC grade from Fisher Scientific (Pittsburgh, PA, USA). All mycotoxin standards (aflatoxins B_1, B_2, G_1, G_2, and a combined standards mixture, and fumonisin B_1) and other chemicals were obtained from Sigma (St. Louis, MO, USA). Selected samples from each year of the study were examined by liquid chromatography/mass spectrometry to confirm mycotoxin identity and the relative amounts of subtypes of fumonisins B_1, B_2, B_3, B_4 and C_4 and aflatoxins B_1 and B_2. Liquid chromatography/mass spectrometry analyses were carried out as described in detail by Abbas et al. (2002). Aflatoxins were determined by liquid chromatography/mass spectrometry with atmospheric pressure chemical ionization and selected ion monitoring (m/z 313 and 315) mode on a Finnigan Mat LCQ system (ThermoQuest, San Jose, CA, USA) equipped with a 5 cm × 4.6 mm i.d. packed with 5μ reversed phase C_{18} HPLC column (Supelco, Bellefonte, PA, USA). Corn samples were extracted using Association of Official Analytical Chemists Method 979.18 (Shotwell and Holaday, 1981) as modified by Abbas et al. (2002). The HPLC mobile phase contained 1% acetic acid plus 8–72% methanol in water. Gradient elution of aflatoxins was done by keeping methanol at 8% for 1 min, increasing to 40% in 19 min, holding at 40% for 5 min, and then increasing to 72% in 5 min. Fumonisins were determined by liquid chromatography/mass spectrometry with electron spray ionization (Abbas et al., 2002) in selected ion monitoring mode at m/z 722, 706, 690, 690 and 676 for fumonisins B_1, B_2, B_3, B_4 and C_4, respectively. Ground corn samples (10 g) were extracted with 30 mL methanol/water (3:1, v/v) in a capped 50-mL centrifuge tube shaker for 1 h, and subjected to cleanup by the method of Thiel et al. (1993). The same HPLC column was used as for aflatoxins, except with a mobile phase of 1% acetic acid plus 8–64% methanol in water. Gradient elution of fumonisins was done by holding the methanol concentration at 8% for 1 min, followed by increasing methanol concentration to 64% in 19 min and holding the methanol concentration at 64% for 10 min. The percentage of each aflatoxin and fumonisin sub-type in a sample was calculated as follows: the area under the curve for each toxin subtype was calculated as a percentage of the total areas under the curves for all sub-types of that toxin detected in the sample.

2.6. HPLC analysis of aflatoxins and fumonisins

The analytical methods used to analyze these toxins were described in detail by Abbas et al. (2002). Briefly, HPLC analysis of aflatoxins used a modification of the method of DeVries and Chang (1982), on a Waters 717 plus autosampler with detection by a Waters 474 scanning fluorescence detector set at 0.3 for sensitivity, 365 nm for excitation, 440 nm for emission and using 12 mm slit width values. Samples and standards were programmed to inject and run for 20 min each at 2.5 mL/min through a Supelco 250 × 4.6 mm i.d. Discovery C_{18} column protected by a 20 × 4.0 mm, C_{18} i.d. pre-column, with both columns containing 5 μm beads. The mobile phase was isocratic acetonitrile:water:acetic acid (24:76:1) delivered by a Waters 510 pump. Sample data was collected using PC-Chrom Software (H & A Scientific, Greenville, NC, USA). HPLC analyses of fumonisins were conducted on the same instrument, except that the excitation wavelength of the fluorescence detector was set at 345 nm and the emission wavelength at 440 nm, and a fumonisin-dedicated column of the same type as for aflatoxin was used with methanol:sodium phosphate buffer (pH 3.33), 80:20 as mobile phase at a 1 mL/min flow rate for 20 min. Samples were derivatized in the autosampler before application to the column. *o*-Phthaldialdehyde/*β*-mercaptoethanol reagent was transfered from a source vial to a designated sample vial 10-min before the samples were injected onto the column. The wait period is critical, because derivative formation takes 10 min, but derivatives degrade rapidly after that time. Standards of fumonisin B_1 and B_2 (0.5–10 ng each) were treated in the same manner. The clean-up and HPLC analysis of fumonisins were based on the method of Thiel et al. (1993) as modified by Abbas et al. (2002). A detection limit of 0.5 μg/kg was obtained for each standard aflatoxin, and 0.5 mg/kg for each standard fumonisin based on a signal-to-noise ratio $\geqslant 3$ to 1. A trace was defined as a readily discernable

peak (signal-to-noise ratio $\geqslant 1.5$) that is below the limit of detection.

2.7. Statistical analysis

Relationships of aflatoxin levels to fumonisin levels and to heat-related factors were analyzed by correlation analysis and regression analysis. Comparison of mycotoxin levels observed under various conditions was also evaluated for significance by Student's unpaired *t*-test assuming equal variances. In all cases, the statistical package included in Microsoft Excel 97 software was used.

3. Results and discussion

Fungal ear rot and ear damage in the corn hybrids from 1998 recorded at the time of harvest are shown in Table 1. Levels of insect damage, Fusarium ear rot and total ear rot were significantly lower ($P<0.01$, Student's *t*-test) in Bt than in non-Bt hybrids. Among all hybrids, regression analysis indicated that Fusarium ear rot was strongly related to insect damage ($r^2 = 0.895$, $P<0.0001$), and that Aspergillus ear rot also related significantly with insect damage ($r^2 = 0.205$, $P<0.05$). The two types of ear rot correlated significantly with each other ($r = 0.454$, $P<0.05$).

In 1998, 100% of kernel corn samples had detectable amounts of both aflatoxins and fumonisins that exceeded the regulatory action levels of 20 ppb aflatoxin (United States Food and Drug Administration, 2000) and 2 ppm for fumonisin (United States Food and Drug Administration, 2001) when analyzed by ELISA or HPLC (Table 2). Total aflatoxins ranged from 21 to 699 ppb, and total fumonisins ranged from 22 to 86 ppm. Corn samples from all hybrids, including all Bt hybrids, were contaminated with aflatoxins and fumonisins (Table 2). The values obtained by ELISA correlated significantly with the total values obtained by HPLC by regression analysis for both aflatoxin levels ($r^2 = 0.252$, $P<0.05$) and fumonisin levels ($r^2 = 0.794$, $P<0.001$). Because the values obtained by the two methods were not significantly different ($P = 0.4$, Student's *t*-test), it was concluded that the more convenient ELISA method yielded reliable results.

Table 1
Physical properties and disease in Arkansas corn hybrids in 1998

Corn hybrid	Ear length (cm)	% Insect damage	Ear rot (%)		Total
			Aspergillus	Fusarium	
Conventional hybrids					
AT787	16.3	6.4	0.05	4.7	4.8
DK363RR (Exp)	14.2	4.6	0.15	3.2	3.4
DK626	13.0	8.4	0.65	6.2	6.9
DK687	13.5	6.2	0.19	3.8	4.0
F5510A	15.7	5.6	0.05	3.1	3.1
P3223	15.6	4.6	0	3.5	3.5
P3245	14.7	8.8	1.43	5.7	7.2
P3245IR	15.5	8.6	0.14	4.9	5.1
P33G26	15.2	6.4	0	3.9	3.9
P3335	14.7	10.5	0.67	5.7	6.4
P3394	14.5	3.3	0.03	2.0	2.0
P3395IR	12.2	5.7	0	2.7	2.7
TR1157	16.5	5.4	0.02	3.6	3.6
TV2090	14.5	6.5	0.28	4.2	4.4
TV2100	14.7	13.2	0.30	8.2	8.5
TV2140	17.3	7.4	0.12	5.4	5.6
TV2543	16.3	6.7	0.05	4.9	5.0
Mean ± SE	15.0 ± 0.3	7.0 ± 0.6	0.24 ± 0.09	4.5 ± 0.4	4.7 ± 0.4
Bt corn					
P31B13	16.0	2.8	0.1	1.7	1.8
P33V08	13.2	2.6	0.15	2.3	2.4
P33Y09	13.5	3.3	0	3.1	3.1
P33A14	15.7	2.8	0.38	2.0	2.4
Mean ± SE	14.6 ± 0.7	2.8 ± 0.1*	0.16 ± 0.08	2.3 ± 0.3*	2.4 ± 0.3*
Total					
Mean ± SE	14.9 ± 0.3	6.2 ± 0.6	0.23 ± 0.07	4.0 ± 0.4	4.3 ± 0.4

*Bt corn levels were significantly lower than conventional hybrid levels, $P<0.01$, by Student's unpaired *t*-test assuming equal variances.

Consequently, only ELISA was used to quantitate total mycotoxin levels in the remaining years of the study.

Average mycotoxin contamination in all conventional hybrids studied was reduced dramatically from 215±49 in 1998 to 26.6±17.5 ppb in 1999 for total aflatoxins (87.6% reduction, $P<0.0001$, Student's *t*-test), and from 48.1±4.2 in 1998 to 3.3±0.6 ppm in 1999 for total fumonisins (93.1% reduction, $P<0.0001$, Student's *t*-test) (Table 3). Among those hybrids planted in both 1998 and 1999 (indicated with asterisks in Tables 2 and 3), the average total aflatoxin level was reduced from 246±99 ppb in 1998 to <5 ppb in each in 1999, and the average total fumonisin level was reduced from 42.9±6.4 ppm in 1998 to 2.7±0.5 ppm in 1999 (93.7% reduction, $P<0.0001$, Student's *t*-test). The observed levels of fumonisins in the hybrids planted both years did not correlate significantly with each other between 1998 and 1999 ($r=0.31$, $P=0.50$). In 1999, total aflatoxins ranged from <5 ppb in many of the hybrids to 255.3 ppb, and total fumonisins ranged from 0.3 to 8.1 ppm (Table 3). The amounts of both aflatoxins and fumonisins were lower in Bt corn than in non-Bt corn in 1999 (Table 3), but only the Bt-associated reduction in fumonisins was significant ($P<0.05$, Student's *t*-test).

In 2001, the average aflatoxin level in corn harvested at two locations in Arkansas, Cotton Branch Experiment Station and Pine Tree Experiment Station, was again much lower than in 1998 ($P<0.001$, Student's *t*-test), but not significantly different from aflatoxin levels at the same location in 1999 (Student's *t*-test). Total aflatoxins in corn harvested from both locations ranged from <5 ppb in 85% of hybrids to 70.2 ppb (Table 4). However, fumonisin contamination in corn harvested in both locations was found to be much higher than 1999 ($P<0.001$, Student's *t*-test), ranging from 17.6 to 58.4 ppm (Table 4), but significantly lower than levels found in corn harvested in 1998 ($P<0.01$, Student's *t*-test). Fumonisin levels at Cotton Branch Experiment Station were significantly higher than at Pine Tree Experiment Station ($P<0.001$, Student's *t*-test), but differences between aflatoxin levels were not significant.

Table 2
Aflatoxin and Fumonisin levels Arkansas corn in 1998

	ELISA		HPLC					
	Aflatoxins (ppb)	Fumonisins (ppm)	Aflatoxins (ppb)			Fumonisins (ppm)		
			AFB_1	AFB_2	Total	FB_1	FB_2	Total
Conventional hybrids								
AT787	248	51.5	178	29	207	40.0	2.0	42.0
DK363RR (Exp)	133	35.0	77	2	79	22.7	1.0	23.7
DK626	417	49.0	502	10	512	60.5	5.9	66.4
DK687	21	34.8	28	0	28	35.8	2.6	38.4
F5510A	58	28.0	63	0	63	25.2	6.5	31.7
P3223[a]	94	39.0	99	0	99	30.6	2.0	32.6
P3245[a]	699	57.8	641	12	653	47.9	2.4	50.3
P3245IR	259	73.5	202	9	211	70.0	5.9	75.9
P33G26[a]	40	54.3	27	0	27	50.5	3.1	53.6
P3335[a]	527	66.5	408	8	416	65.1	10.0	75.1
P3394[a]	71	25.3	233	4	237	10.4	0.0	10.4
P3395IR	33	34.5	36	0	36	13.9	0.0	13.9
TR1157	105	39.0	241	3	245	30.3	0.0	30.3
TV2090	173	26.5	172	4	176	12.1	0.5	12.6
TV2100	190	78.5	395	5	400	75.0	10.5	85.5
TV2140	371	74.5	378	10	388	75.0	10.1	85.1
TV2543	74	50.8	73	0	73	45.0	2.0	47.0
Mean±SE	215±49	48.1±4.2	221±45	5.6±1.8	227±46	41.8±5.3	3.8±0.9	45.6±6.0
Bt hybrids								
P31B13[a]	225	22.8	204	5	209	25.3	9.0	34.3
P33V08[a]	66	34.5	85	3	88	40.0	9.0	49.0
P33Y09	70	46.3	54	0	54	30.0	1.0	31.0
P33A14	428	49.3	430	3	433	42.4	1.8	44.2
Mean±SE	197±85	38.2±6.1	193±85	2.±1.08	196±86	34.4±4.1	5.2±2.2	39.6±4.2
Total corn hybrids								
Mean±SE	205±41	46.3±3.6	179±41	5.1±1.5	221±40	40.4±4.4	4.1±0.8	44.4±4.9

[a]This hybrid was also planted in 1999. *Abbreviations*: ELISA = enzyme-linked immunosorbant assay, HPLC = high-performance liquid chromatography, SE = standard error.

Table 3
Aflatoxin and Fumonisin levels Arkansas corn grown in 1999

Corn hybrid	Aflatoxins (ppb)	Fumonisins (ppm)
Conventional hybrids		
AsRX770YG	<5.0	6.8
N63-G7	255.3	4.9
N83-N5	89.0	5.8
P32P76	<5.0	2.0
P33R87	<5.0	1.5
P34B23	32.1	8.1
P32K61	<5.0	1.8
P3335[a]	<5.0	2.7
P33R88	<5.0	0.3
P3245[a]	<5.0	4.6
P33G26[a]	<5.0	2.7
P33K81	<5.0	1.3
P3223[a]	<5.0	1.6
P32P75	22.4	1.3
P3394[a]	<5.0	4.4
Mean±SE	26.6±17.5	3.32±0.60
Bt hybrids		
AsRX799Bt	<5.0	3.3
C7821Bt	<5.0	2.0
DK595BtY	7.3	4.4
DK679BtY	<5.0	3.0
DK626Bt	<5.0	1.0
FFR769Bt	<5.0	2.2
N75-90Bt	<5.0	1.5
P32K62Bt	<5.0	1.5
P33V08[a]	<5.0	1.0
P31B13[a]	<5.0	2.0
TVX 21681 Bt	5.9	1.3
TVX 21680 Bt	5.7	1.7
TVX 21483 Bt	25.1	2.4
Tr1866 Bt	6.7	2.0
Mean±SE	3.6±1.8	2.09±0.25*
Total corn hybrids		
Mean±SE	15.5±9.2	2.73±0.35

An enzyme-linked immunosorbant assay (ELISA) method was used to determine the above results. Limits of detection: Aflatoxin <5 ppb, Fumonisin <1 ppm. Values below the limit of detection are treated as zero in means and statistical analysis.
*Bt corn fumonisin levels were significantly lower than conventional hybrid levels, $P<0.05$, by Student's unpaired t-test assuming equal variances.
[a]This hybrid was also planted in 1998.

Table 4
Aflatoxin and Fumonisin levels in corn grown during 2001 at two Arkansas locations

Corn hybrid	Pine Tree Experimental Station		Cotton Branch Experiment Station	
	Aflatoxin (ppb)	Fumonisin (ppm)	Aflatoxin (ppb)	Fumonisin (ppm)
Conventional hybrids				
P3245	<5	35.5	8.0	56.8
P3394	<5	31.3	37.5	34.9
P3223	<5	20.0	<5	29.5
P33R87	<5	31.7	<5	55.1
P32P76	70.2	21.9	<5	58.4
P34B23	<5	24.4	<5	50.6
DKD	<5	17.6	<5	41.0
Mean±SE	10.0±10.0	26.1±2.6	6.5±5.3	46.6±4.3
Bt Hybrids				
P33VO8	<5	40.5	<5	52.0
P31B13	<5	20.2	<5	56.5
Mean±SE	<5	30.4±10.2	<5	54.3±2.3
Total corn hybrids				
Mean±SE	7.8±7.8	27.0±2.7*	5.1±4.2	48.3±3.5*

Enzyme-linked immunosorbant assay (ELISA) methods were used to determine the above results.
*Fumonisin levels were significantly lower at Pine Tree Experimental Station than Cotton Branch Experiment Station, $P<0.01$, by Student's unpaired t-test assuming equal variances.

Because ELISA assays measure total aflatoxins and total fumonisins, not individual subtypes, an additional study was conducted to determine if mycotoxin subtype patterns were similar throughout the period studied. Ten corn samples representing high, low and intermediate mycotoxin contamination levels were selected from 1998, five from 1999, and four from the 2001 growing seasons. They were analyzed by liquid chromatography/mass spectrometry for relative levels of aflatoxins B_1 and B_2 using chemical ionization, and in separate runs for relative levels of fumonisins B_1, B_2, B_3, B_4, and C_4 using electrospray ionization. Amounts of each aflatoxin and fumonisin subtype shown in Table 5 were calculated by multiplying the fraction of total area under the curve corresponding to it on the liquid chromatography/mass spectrometry tracing by the total amount of aflatoxin or fumonisin measured in the same extracts using ELISA. Aflatoxin B_1 was observed in 84.2% of hybrids, and it was the predominant toxin subtype in all samples. Aflatoxin B_2 was observed only in 1998 and only in the two hydrids (10.8% of hybrids examined) producing the largest amounts of total aflatoxins. Fumonisins were found in all years and all samples examined. Fumonisin B_1 was the predominant subtype in all samples examined except one (5.4% of hybrids examined). Fumonisins B_1, B_2, B_3, and B_4 were found in all samples examined except in two (10.8%) with low total fumonisin levels, in which they were present in trace amounts (i.e., discrete peaks, but less than the 3:1 signal-to-noise ratio used to define the limit of detection). Fumonisin C_4 was present in 48% of samples, but the frequency varied with the year, being detectable in all samples in 1998, in one sample (20%) in 1999 and not detected in 2001. Fumonisin A_4 was detected in trace amounts by liquid chromatography/mass spectrometry in 20% of samples, all of which contained readily detected fumonisin C_4 (data not shown).

Weather conditions influence aflatoxin and fumonisin contamination of corn (Payne, 1992), although *Fusarium* spp. are found in a wider range of climate conditions. Heat stress during the period when the

Table 5
Levels of various aflatoxin and fumonisin subtypes in Arkansas corn samples determined by liquid chromatography/mass spectrometry methods

Year and corn hybrid	Aflatoxin levels (ppb)			Fumonisin levels (ppm)					
	Total[a]	AFB_1	AFB_2	Total[a]	FB_1	FB_2	FB_3	FB_4	FC_4
1998									
TV2100	98	98	ND	73	38	8.3	6.7	20	0.30
AT787	117	117	ND	47	6.3	22	13	4.6	0.84
DK363RR	297	297	ND	35	23	6.8	3.5	1.3	0.17
DK626	1,190	1120	69.1	53	33	12.1	5.3	2.0	0.30
P31B13	669	633	36.1	38	23	10	3.1	1.2	0.15
P3245IR	6	6	ND	64	37	20	4.5	2.3	0.37
P33A14	59	59	ND	33	21	7.3	3.0	1.9	0.22
P3335	132	132	ND	93	55	21	12	4.4	0.66
TR1157	66	66	ND	41	27	8.8	3.4	1.8	0.28
TV2140	69	69	ND	71	40	19	7.9	4.3	0.51
1999									
N63-G7	428	428	ND	8.6	4.4	0.98	0.79	2.40	ND
N83-N5	218	218	ND	8.0	5.7	1.5	0.45	0.35	ND
P34B23	48.3	48.3	ND	16.5	11.0	3.24	1.42	0.73	0.10
P3223	ND	ND	ND	0.1	0.07	0.02	0.01	ND	ND
P32P75	39.8	39.8	ND	1.9	1.3	0.40	0.15	0.06	ND
2001									
P3245	ND	ND	ND	88	49	7.7	25	6.1	ND
P32P7	525	525	ND	9.7	5.8	0.77	2.6	0.48	ND
P33R8	ND	ND	ND	3.1	2.0	0.21	0.85	ND	ND
P34B2	18.5	18.5	ND	37	20	3.8	10	3.5	ND

ND = not detected.
[a]Total mycotoxin values were determined by enzyme-linked immunosorbant assay (ELISA). Values for individual aflatoxin and fumonisin subtypes were calculated by multiplying the total value by the corresponding fraction of the total area under the curve of the liquid chromatography/mass spectrometry tracing.

kernel is developing (i.e., between silking and blacklayer, which is May–July in Arkansas), particularly nighttime temperatures above 20 °C (Payne, 1992; Abbas et al., 2002) is a major factor in mycotoxin contamination. In 1998, Arkansas experienced unusually high temperatures (Table 6), particularly during May and June, providing ideal conditions for production of large amounts of both fumonisins and aflatoxins. In contrast, temperatures in 1999 and 2001 were only modestly above normal. Arkansas received much lower than normal rainfall in 1998 (Table 6), but in the present study irrigation was used to minimize the effect of drought stress. Similarly, fertilization was used to minimize nutrient stress, and optimal planting and weed control methods were used to minimize population stress, leaving heat as a major uncontrolled source of stress, although additional unrecognized sources of stress may have been present. There was a close positive correlation between total growing season degree growing units above the 30-year norm and average aflatoxin levels in conventional hybrids ($r = 0.996$) and in all hybrids studied ($r = 0.999$), and a less close but positive correlation with average fumonisin levels in all hybrids studied ($r = 0.655$) during the 3 years. However, it will be necessary to study the effect of heat stress on mycotoxin levels in corn over a longer time period to establish the significance of this correlation.

It has been suggested that there is a negative relationship between *Aspergillus* spp. and *Fusarium* spp. infection in corn (Marin et al., 1998b). If such a negative relationship exists, one would expect that aflatoxin levels would correlate negatively with fumonisin levels. However, in the present study, a significant positive correlation between aflatoxin and fumonisins levels was observed for all hybrids studied in 1998 ($r = 0.502$, $P < 0.05$), in 1999 ($r = 0.369$, $P < 0.05$) and in all years combined ($r = 0.518$, $P < 0.001$), but not in 2001 at either Cotton Branch Experiment Station and Pine Tree Experiment Station. Even among Bt hybrids, in which insect-mediated infection might be expected to be reduced, a positive correlation between aflatoxin and fumonisin levels ($r = 0.298$) was observed in all years combined, although it was not statistically significant. Thus, the results in this study, along with others (LaPrade and Manwiller, 1977; Lillehoj et al., 1977; Chamberlain et al., 1993; Widstrom et al., 1994), indicate that natural infection with *Fusarium* spp. does not appear to protect against production of aflatoxin. The observation that conditions in 1998 favored production of high levels of both aflatoxins and

Table 6
Summary of mean temperatures and precipitation observed at the National Climate Data Center, Mariana, Arkansas, during the growing seasons (May–July) studied

Year	Month	T_{max} (avg. max. air temp, °C)	T_{min} (avg. min. air temp, °C)	Total DD20[a]	Cumulative rainfall (cm)
1998					
	May	29	18	+108.5	3.6
	June	33	23	+240	4.1
	July	34	24	+279	6.7
Growing season total				+627.5	14.4
1999					
	May	27	16	+46.5	8.3
	June	30	21	+165	12.0
	July	33	23	+248	8.8
Growing season total				+459.5	29.1
2001					
	May	28	17	+77.3	10.2
	June	30	20	+150	8.8
	July	32	23	+232.5	5.5
Growing season total				+460	24.5
30-year Norm					
	May	27	15	31	13.6
	June	31	20	+165	11.2
	July	33	22	+232.5	9.7
Growing season total				+428.5	34.5

[a]DD20 = degree growing units = $(T_{max} + T_{min})/2 - 20$ °C. Total DD20 is the sum of DD20s over the growing period.

fumonisins, whereas conditions in 2001 favored high fumonisins but low aflatoxins, suggests that it may not be possible to control both mycotoxins with the same cultural practices. Further research is planned to better understand the factors which control mycotoxin levels in corn.

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Disclaimer

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